Official journal website:
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Amphibian & Reptile Conservation
14(1) [General Section]: 119–131 (e225).

First record of *Raorchestes longchuanensis* Yang and Li, 1978 (Anura: Rhacophoridae) from northeastern Bangladesh suggests wide habitat tolerance

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Abstract.—Raorchestes **is a genus of small bush frog characterized by an absence of vomerine teeth, direct development without free swimming larvae, and a transparent gular pouch while calling. During a larger study on canopy fauna in the northeastern region of Bangladesh, five specimens of a small bush frog were collected from Satchari National Park in June and July 2017. This species was confirmed as *Raorchestes longchuanensis* using both morphometric and genetic analyses. Although this species was originally described from Yunnan, China, the authors speculated that it may be found in neighboring countries adjacent to the original records, including northern Myanmar, Thailand, Laos, and Vietnam. However, the current finding suggests that the species could be more widespread and resilient, spanning westwards through to northeastern India and Bangladesh. Data are also provided on coloration, habitat, natural history, and vocalizations of this little-known species. Although the species is designated Least Concern according to IUCN, more comprehensive studies should be undertaken to better understand its biology and population status to aid in a more comprehensive global conservation assessment.**

Keywords. Amphibian, Asia, bush frog, DNA, range extension, Satchari National Park

Citation: Al-Razi H, Maria M, Hasan S, Bin Muzaffar S. 2020. First record of *Raorchestes longchuanensis* Yang and Li, 1978 (Anura: Rhacophoridae) from northeastern Bangladesh suggests wide habitat tolerance. *Amphibian & Reptile Conservation* 14(1): 119–131 (e225).

Received: 15 February 2019; **Accepted:** 10 August 2019; **Published:** 23 March 2020

Introduction

Amphibians around the world are declining due to a variety of factors including habitat loss, competition with invasive species, emerging infectious diseases, and climate change (Blaustein et al. 2010; Fisher et al. 2009; Gray et al. 2009; Hayes et al. 2010; Pounds et al. 2006; Whittaker et al. 2013). Globally, nearly one-third of known amphibian species are threatened with extinction, and at least 42% of known amphibian species are experiencing population losses (Whittaker et al. 2013). These problems are acute in tropical Asia where large swaths of forest and wetland areas have already been replaced with agricultural land or urban environments, while existing patches are under constant pressure from human expansion. Thus, forest biodiversity within the tropical and subtropical belts has been the focus of major efforts in biodiversity conservation worldwide due to their recognized importance in climate stabilization, economic value (e.g., timber production or non-timber forest products), and to a lesser extent ecotourism potential (Muzaffar et al. 2011). Tropical forests in South Asia have been classified variously into a range of tropical moist, tropical evergreen, and several other forest types based on the composition of vegetation (Champion and Seth 1968).

Recent work using phylogeographic approaches suggests that South Asian and Indo-Pacific forest types exhibit more compositional overlap than previously imagined (Slik et al. 2018). Incidentally, many of these areas are just beginning to be adequately explored for amphibian biodiversity and many species of forest-associated amphibians are being described (Biju and Bossuyt 2009; Biju et al. 2010a,b, 2011, 2014; Hasan et al. 2014a; Mahony 2011; Mahony et al. 2013, 2018; Purkayastha and Matsui 2012). Not surprisingly, many of these newly discovered species are already under some form of threat and could be heading towards extinction (IUCN 2015). Bangladesh hosts at least 64 species of anurans (Khan 2015), with several recently discovered new species (Hasan et al. 2012, 2014a; Howlader 2011; Howlader et al. 2015, 2016) and new country records (Hasan et al. 2010; Khan 2001; Mahony and Reza 2007a,b, 2008; Reza 2008), and several others are likely

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to be added to the list in the near future (Khan 2015).

Raorchestes Biju, Shouche, Dubois, Dutta, and Bossuyt (2010) is a genus of small-sized frogs with direct development in the Rhacophoridae family. A total of 62 *Raorchestes* species are known from India, Nepal, Bangladesh, Myanmar, Thailand, Laos, Southern China, Vietnam, Cambodia, and West Malaysia (Frost 2019). Although four species are reported from Northeast India, only two species of this genus are found in Bangladesh (Frost 2019; IUCN 2015). Of the 62 *Raorchestes* species, 58 are from South Asia and only four species, including *Raorchestes longchuanensis* (Yang and Li 1979), occur in Southeast Asia and southern China (Frost 2019).

In this study, *Raorchestes longchuanensis* is reported from the north-eastern region of Bangladesh, based on observations made during a survey conducted as part of a larger study on canopy fauna in this region. Forests in the northeastern regions are considered to be tropical moist deciduous or tropical semi-evergreen forests (Champion and Seth 1968) and are located in the south and southwest of the Indian states of Meghalaya, Assam, and Nagaland, along with comparable forest types such as tropical moist deciduous and subtropical broad-leaved hill forests (Champion and Seth 1968). The objectives of this study were (i) to diagnose the presence of this frog species hitherto reported only from southern China; (ii) to provide morphometric, acoustic, and natural history data on this species; and (iii) to provide comments on its biogeography.

Methods

Study Area

Surveys were conducted in Satchari National Park (SNP), located in Habiganj District, Sylhet in northeastern Bangladesh. The park encompasses an area of about 243 ha (Mukul et al. 2010) and is located on Raghunandan hill, under Paikpara Union, Chunarughat Upazilla. The topography of this area is undulating with slopes and hillocks, ranging from 10–150 m in elevation. The climate is mainly tropical with high rainfall (300–800 mm) concentrated during the monsoon from June to September, with temperatures ranging from 25–32 °C. In the remaining dry period of the year, the region experiences occasional, low rainfall and lower temperatures of 12–25 °C (Mollah et al. 2004). The total annual average rainfall is 4,162 mm (Mollah et al. 2004). A number of small, sandy-bedded streams drain the forest during the rainy season. The maximum and minimum temperatures of the area are 32 °C and 12 °C, respectively, and the relative humidity fluctuates between 74% and 90% (Mollah et al. 2004).

Field Survey

Five specimens were collected in June and July 2017. Only acoustic searches were used to locate the calling aggregations (Heyer et al. 1994). The temperature, humidity, habitat, perch height, and behavioral activities were recorded after encounter. All individuals collected were males since they were located based on their calls. No female individuals were found. The location of each collected specimen was recorded by a GPS device (Garmin eTrex 10), and temperature and humidity were recorded with a digital thermometer and hygrometer, respectively. Video footage of a calling male individual was also recorded using a DSLR camera (Nikon d7200 with 55–300 mm Vr lens). Five adult male individuals were collected from two different field surveys, fixed in formalin, and preserved in 70% ethanol. A small amount of thigh muscle was collected (as described below) from four specimens, and these tissue specimens were preserved in 70% ethanol. Specimens were deposited in the Museum of the Department of Zoology, Jagannath University, Dhaka, Bangladesh (reference numbers: JnUZool-A0117 to JnUZool-A0517).

Morphometrics

The following morphometric measurements of the specimens were taken before preservation with a digital caliper (to the nearest 0.1 mm): snout-vent length, from tip of snout to vent (SVL); head length, distance between tip of snout and rear of mandible (HL); head width, at angle of jaw (HW); eye diameter, horizontal diameter of eye (ED); tympanum diameter, maximum diameter of tympanum (TD); eye-nostril distance, distance between anterior canthus of eye and nostril (END); snout length, from anterior canthus of eye to tip of snout (SL); inter orbital distance, least distance between proximal edges of upper eyelids (IOD); internarial distance, least distance between nostrils (IND); upper eyelid width, maximum transverse distance of upper eyelid measured from inner edge to outer edge (UEW); eye-tympanum distance, least distance between eye and tympanum (ETD); thigh length, distance from middle of vent to knee (THL); shank length, distance between knee and heel (SL); foot length, from base of inner metatarsal tubercle to tip of Toe IV (FL); hand length, from base of outer palmar tubercle to tip of Finger IV (HAL); width of 1st to 4th finger disks, measured at the widest point on the finger disk (FinDW); lengths of 1st to 4th fingers, from base of palm to tip of respective finger (FinL, F-I to F-IV); lengths of 1st to 5th toes, from inner metatarsal tubercle region to tip of respective toe (ToeL, T-I to T-V); and width of 1st to 5th toe disks, greatest horizontal distance between edges of Toe I disks (ToeDW).

DNA Extraction and Amplification

The procedure described in Vences et al. (2012) was followed for DNA extraction. Approximately 1.5 mm^2 of thigh muscle tissue was excised from each specimen for extraction. The PCR amplification and sequencing of the

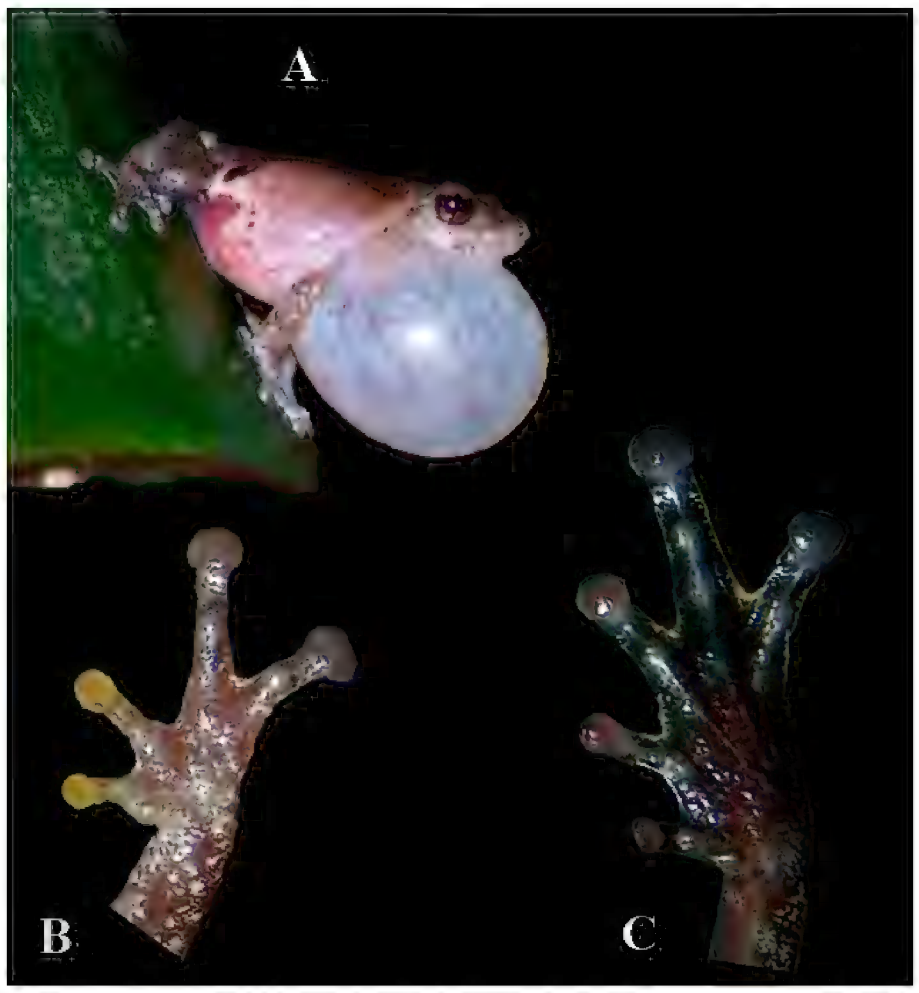

Fig 1. External features of *Raorchestes longchuanensis*. (**A**) Calling position of an adult male individual where transparent gular sack is prominent; (**B**) Ventral aspect of hand with rounded, disked fingertips; (**C**) Ventral aspect of foot with rounded disked, reddish toe tips.

16S rRNA gene were performed following Palumbi et al. (1991) and Bossuyt et al. (2004), respectively. Primers of 5'-GCCTGTTTATCAAAAACAT-3' (16Sar-L) and 5'-CCGGTCTGAACTCAGATCACGT-3' (16Sbr-H) as forward and reverse primers for 16S (Palumbi et al. 1991), were used for this study. PCR amplifications were performed in a 20 µl reaction volume with the following cycling conditions: an initial denaturing step at 95 °C for 3 min; 40 cycles of denaturing at 95 °C for 30 s, annealing at 50 °C for 30 s, and extending at 72 °C for 45 s; and a final extending step of 72 °C for 5 min. The amplified products were sent to 1st Base Laboratories, Malaysia, for sequencing. The sequences were checked manually using the program Chromas lite 2.01 (http://www.technelysium.com.au/chromas_lite.html). The sequences have been submitted to GenBank (Accession nos: MH699074, MN193412, MN193413, and MN193414).

To infer the phylogenetic position of the current species, homologous sequences for 16S rRNA genes of 24 species were downloaded from the NCBI GenBank database (Table 1). Sequences were aligned using the MUSCLE tool in MEGA X (Kumar et al. 2018), and alignments were checked visually and corrected manually when necessary. Alignment gaps were treated as missing data. The best fit model for nucleotide substitution was selected from 24 models available in MEGA X (Kumar et al. 2018) based on the minimum Akaike Information Criterion (AIC) value (Posada and Crandall 2001). Maximum Likelihood was performed in MEGA X. The general time reversal nucleotide substitution model with gamma distribution with Invariant Sites (GTR+G+I), obtained as a best fit model in the model test (AIC = 3932.32, lnL = -1903.84), was used for constructing the phylogenetic tree based on the maximum likelihood method by using 1,000 bootstrap replicates in MEGA X (Kumar et al. 2018). Interspecific and intraspecific mean uncorrected pairwise distances were computed in MEGA X (Kumar et al. 2018).

Call Recording and Analysis

The call of a single male individual (JnUZool- A0317) was recorded with a DSLR camera (Nikon d7200 with 55–300 mm VR lens) on 2 August 2017. The sound was extracted from the video clip by using Windows Movie Maker 2012 software. The microphone was approximately 1–1.5 m away from the calling male. Air temperature and humidity were taken with a digital hygrometer. Call analysis used Raven Pro Ver. 1.5 (Charif et al. 2010; Bioacoustics Research Program 2011). Measured parameters were call-group duration, inter-call group interval, duration of intervals between pulses, call duration, and call rate of a call bout comprised of nine call groups.

Results

First Observation

During the surveys, this species was observed for the first time on 5 June 2017. The day was cloudy and after a rainfall before dusk (1810 h to 1840 h) the surveyors heard several *tik-tik-tik-tik* calls from the bamboo bushes, not very far from the narrow road of the SNP visitor's watch tower. The bamboo bushes were entered and the search for the frogs commenced. As there was still day light, the search for the frog was conducted without any light. After more than 20 min an adult male individual was found on a bamboo tree. The male was calling continuously while keeping its anterior part angled downwards (Fig. 1A). The position of the frog was 1.5 m above the ground. The individual was observed for a few minutes, then collected as a sample specimen.

Species Identification

The species was initially identified as *Raorchestes longchuanensis* based on the limited morphological characters described by Yang and Li (1978) in their original description of the species. *Raorchestes longchuanensis* is a small species in which adult males are 22.9 ± 0.9 mm (n = 5), one-fourth of the toes are webbed, and the disk of the third finger is smaller than the tympanum diameter. This specimen was confirmed as *R. longchuanensis* using the BLAST technique with a sequence similarity of more than 99% (492 of 496 bp). Identification was also confirmed with Maximum

Table 1. 16S rRNA sequences for *Raorchestes* species and *Kurixalus eiffingeri* from GenBank, with accession numbers and localities. SNP = Satchari National Park.

	Species	GenBank accession no.	Locality	Source
01	*R. longchuanensis*	MH699074	SNP, Bangladesh	This study
02	*R. longchuanensis*	MN193412	SNP, Bangladesh	This study
03	*R. longchuanensis*	MN193413	SNP, Bangladesh	This study
04	*R. longchuanensis*	MN193414	SNP, Bangladesh	This study
05	*R. longchuanensis*	GQ285675	China	Yu et al. 2010
06	*R. ghatei*	KF366385	Western Ghats, India	Padhye et al. 2013
07	*R. gryllus*	AB933309	Vietnam	Nguyen et al. 2014
08	*R. shillongensis*	MG980282	Meghalaya, India	Unpublished
09	*R. shillongensis*	MG980283	Meghalaya, India	Unpublished
10	*R. parvulus*	MH590204	Gunung Jerai, Malaysia	Chan et al. 2018
11	*R. menglaensis*	EU924621	Yunnan, China	Yu et al. 2009
12	*R. tuberohumerus*	KP137388	Western Ghats, India	Padhye et al. 2015
13	*R. bombayensis*	KF767502	Castlerock village, Karnataka, India	Padhye et al. 2013
14	*R. bombayensis*	EU450019	Castlerock village, Karnataka, India	Biju and Bossuyt 2009
15	*R. indigo*	KM596557	Western Ghats, India	Vijayakumar et al. 2014
16	*R. ponmudi*	EU450026	Ponmudi, Western Ghats, India	Biju and Bossuyt 2009
17	*R. ponmudi*	KM596576	Ponmudi, Western Ghats, India	Vijayakumar et al. 2014
18	*R. aureus*	KM596540	Western Ghats, India	Vijayakumar et al. 2014
19	*R. montanus*	KM596552	Western Ghats, India	Vijayakumar et al. 2014
20	*R. signatus*	KM596561	Western Ghats, India	Vijayakumar et al. 2014
21	*R. marki*	JX092719	Western Ghats, India	Vijayakumar et al. 2014
22	*R. charius*	KU169985	Karnataka, India	Biju et al. 2016
23	*R. beddomii*	EU449998	Western Ghats, India	Biju and Bossuyt 2009
24	*R. chalazodes*	KJ619641	Western Ghats, India	Das 2015
25	*R. dubois*	JX092668	Koadaikanal, India	Vijayakumar et al. 2014
26	*R. munnarensis*	JX092655	Western Ghats, India	Vijayakumar et al. 2014
27	*R. chromasynchysi*	KM596543	Western Ghats, India	Vijayakumar et al. 2014
28	*Kurixalus eiffingeri*	DQ468673	Okinawa Islands, Japan	Wu et al. 2016

likelihood phylogenetic analyses (Fig. 2) and the intra- and interspecific genetic *p*-distances (0.2–0.3% and 5.0–13.9% average values, respectively, Table 3).

Morphometrics

Snout-vent lengths of the five adult males ranged from 21.4–23.9 mm. Head width was larger than the length of head (HW = 7.8–9.1 mm, HL = 6.30–7.17 mm). The snout was pointed in the dorsal view; snout length was larger than the eye diameter (SL = 2.90–3.12 mm, ED = 2.50–2.86 mm); nostrils were oval and closer to snout tip than to eye; vomerine teeth were absent; eyes were small with a horizontal pupil. Fingers were web-less and finger tips had disks that were rounded. Relative lengths of fingers were as follows: I < II < IV < III (Table 2). The thigh was slightly longer than the tibia (TL = 11.20–12.10 mm, THL = 11.70–12.50 mm); Toe V was longer than Toe III; relative lengths of toes were as follows: I < II < III < V < IV (Table 2). Subarticular tubercles were prominent and rounded; outer metatarsal tubercles were absent while inner metatarsal tubercles were present; and toe tips possessed distinct, rounded disks (Fig. 1B–C).

Coloration

Considerable color variation was recorded in this species, including four different body colors in the specimens that could change in relation to the environment. A distinct or faint ")(" mark was always present on the dorsal surface of the body (Fig. 3). Sometimes a narrow yellow mid-dorsal line was present on the dorsal side (in one of five specimens). Vocal sac was external, single, and transparent with white spotting. The discs of the fingers and toes were reddish, orange, or whitish. There was a pale or prominent brown or black line between the upper eyelids. The dorsum was of a dark chocolate, dark brown, or pale brown coloration in the live specimens. The dorsal coloration and the pale flanks became gray or light brown to dark brown after preservation. Eyelids and tympanic fold were blackish or brown (Fig. 3).

Habitat, Natural History, and Vocalization

The specimens of *Raorchestes longchuanensis* were found in bushes inside the forest, and sometimes near road sides or near human habitations. Usually they were

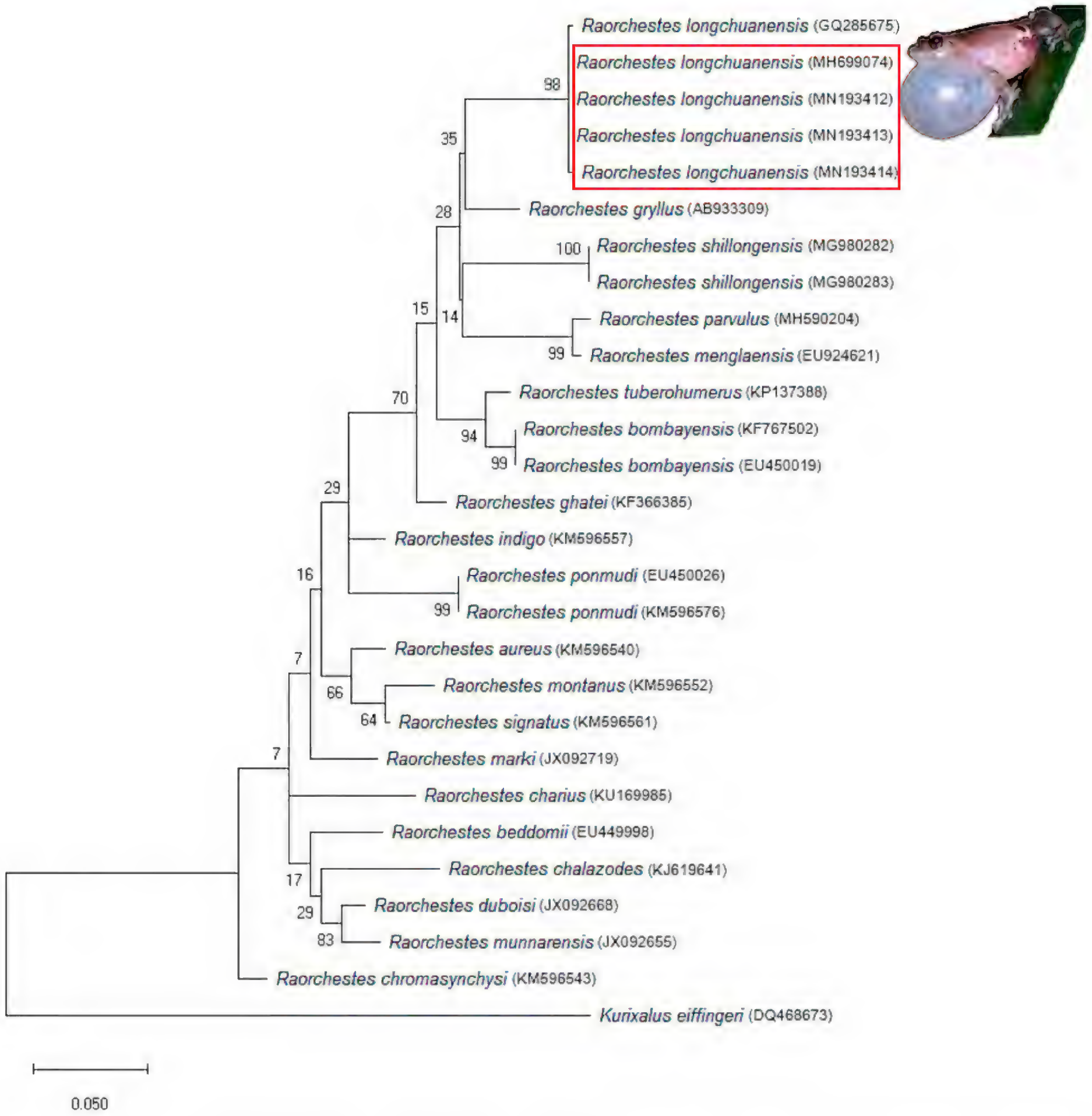

Fig 2. Maximum likelihood phylogenetic tree based on 16S rRNA genes, showing the identity of the specimens as *Raorchestes longchuanensis*. The red box shows the specimens from the present study.

found living amongst the bamboo bushes and Calamus palm bushes (*Calamus* spp.). Individuals usually were perched on leaves and branches of small trees or bamboo bushes. All individuals were found less than 2 m above the ground. *Rarochestes longchuanensis* became active with the onset of rain in the month of April. Breeding males started calling from dusk and continued until 2400 h. During rain and in cloudy environments they became active in the day time.

Advertisement calls occurred in call groups (Fig. 4). The duration of the analyzed call was 20 s. The number of call groups within this call was nine, and the number of pulses varied from three to six. The duration of each call group was 0.526 ± 0.142 s (n = 9) and it varied with the number of calls. The interval between call groups was 1.831 ± 0.403 s (n = 8). Pulse duration was 0.013–0.024 s (0.018 ± 0.003 s, n = 42 pulses), and the duration of intervals between pulses was 0.102–0.163 s (0.124 ± 0.013 s, n = 33 intervals). The lowest frequency was 1.35 kHz and the highest frequency was 4.07 kHz. The overall peak frequency of the calls was 2.76 kHz. The air temperature was 27.4 °C at the time of the call.

Discussion

Several species of frogs have been recorded for the first time or described from Bangladesh in recent years (Asmat 2007; Hasan et al. 2012, 2014a,b; Howlader

Table 2. Morphologic measurements (mm) of *Raorchestes longchuanensis* specimens collected from Satchari National Park, Bangladesh (n = five males).

Characters	Abbreviation	JnUZool-A0117	JnUZool- A0217	JnUZool- A0317	JnUZool- A0117	JnUZool-A0418
Snout-vent length	SVL	22.60	23.90	21.40	23.89	22.52
Head length	HL	6.80	7.17	6.40	7.03	6.80
Head width	HW	8.80	9.10	8.69	9.00	8.00
Eye diameter	ED	2.75	2.86	2.50	2.71	2.66
Tympanum diameter	TD	1.50	1.56	1.43	1.6	1.49
Eye-nostril distance	EN	2.15	2.22	1.88	2.20	1.96
Snout length	SL	3.12	3.10	2.90	3.07	3.03
Interorbital distance	IOD	3.18	3.52	3.28	3.40	3.40
Internarial distance	IND	2.35	2.50	2.25	2.47	2.34
Upper eyelid width	UEW	1.32	1.61	1.25	1.60	1.28
Eye-tympanum distance	ETD	0.72	0.82	0.70	0.75	0.71
Thigh length	THL	12.34	12.50	11.70	12.41	12.26
Tibia length	TL	12.00	12.10	11.20	12.08	12.00
Foot length	FL	8.90	9.56	8.83	9.53	9.03
Hand length	HAL	5.72	6.21	5.69	6.17	6.06
Forearm length	FLL	5.8	6.42	5.61	6.33	6.00
Finger I disk width	Fin1DW	0.50	0.56	0.47	0.56	0.48
Finger II disk width	Fin2DW	0.80	0.85	0.79	0.87	0.80
Finger III disk width	Fin3DW	1.30	1.47	1.26	1.56	1.31
Finger IV disk width	Fin4DW	1.23	1.25	1.18	1.25	1.22
Finger I length	Fin1L	1.61	1.93	1.6	1.92	1.59
Finger II length	Fin2L	2.20	2.38	1.92	2.33	2.17
Finger III length	Fin3L	4.14	4.36	4.04	4.31	4.15
Finger IV length	Fin4L	2.50	2.52	2.35	2.53	2.47
Toe I length	Toe1L	3.24	3.25	2.87	3.32	3.20
Toe II length	Toe2L	4.51	4.56	4.52	4.62	4.50
Toe III length	Toe3L	6.96	6.99	6.93	7.08	6.95
Toe IV length	Toe4L	8.71	8.98	8.69	9.04	8.68
Toe V length	Toe5L	6.35	6.08	6.77	6.96	6.30
Toe I disk width	Toe1DW	0.52	0.58	0.43	0.53	0.50
Toe II disk width	Toe2DW	0.80	0.77	0.74	0.82	0.72
Toe III disk width	Toe3DW	0.88	0.87	0.82	0.90	0.85
Toe IV disk width	Toe4DW	1.18	1.26	1.11	1.30	1.21
Toe V disk width	Toe5DW	0.86	0.98	0.76	1.02	0.87

Fig 3. Color variation in *Raorchestes longchuanensis*. (**A**) Pale brown dorsum with faint ")(" mark; (**B**) Dark brown dorsum with prominent ")(" mark; (**C**) Dark chocolate color dorsum without mid-dorsal line; (**D**) Dark chocolate color dorsal side with narrow yellow mid-dorsal line.

2011; Howlader et al. 2015, 2016; Khan 2015; Mahony and Reza 2007a,b, 2008; Reza 2008; Mahony et al. 2009). This is not surprising since Bangladesh is a small country situated on the boundary of two major ecological realms, with the Indomalayan realm encompassing most of Bangladesh and the beginning of the Palearctic realm to the north (Udvardy 1975). The position of Bangladesh on the edge of these biogeographic zones makes it prone to hosting biota from both realms. These areas have been also recognized as part of the Indo-Burma global biodiversity hotspot, with significant abundance of threatened and regionally endemic amphibians (Sechrest et al. 2002; Jenkins et al. 2013). Until recently, only 49 species of amphibians were officially recorded from Bangladesh (Hasan et al. 2014b). The recent rise in new amphibian records constitutes an almost 65% increase in the anurans of Bangladesh since the late 1990s, suggesting that exploration of the region's amphibians is just beginning (calculated from IUCN 2015; Khan 2015).

The tree frogs of Bangladesh have been particularly under-studied. One species in the genus *Philautus* was originally recorded from Bangladesh (Hasan and Feeroz 2014). This genus was originally considered to be distinguishable from others by the absence of vomerine teeth and its occurrence in South and Southeast Asia (Bossuyt and Dubois 2001). However, further study showed that *Philautus* exhibits diverse morphological traits and a wide variety of life history strategies (Bossuyt and Dubois 2001). *Philautus* from the Western Ghats could be separated from *Pseudophylautus* (Sri Lankan sister clade) and appeared to be distinct from those in northeastern India. This led to the erection of a

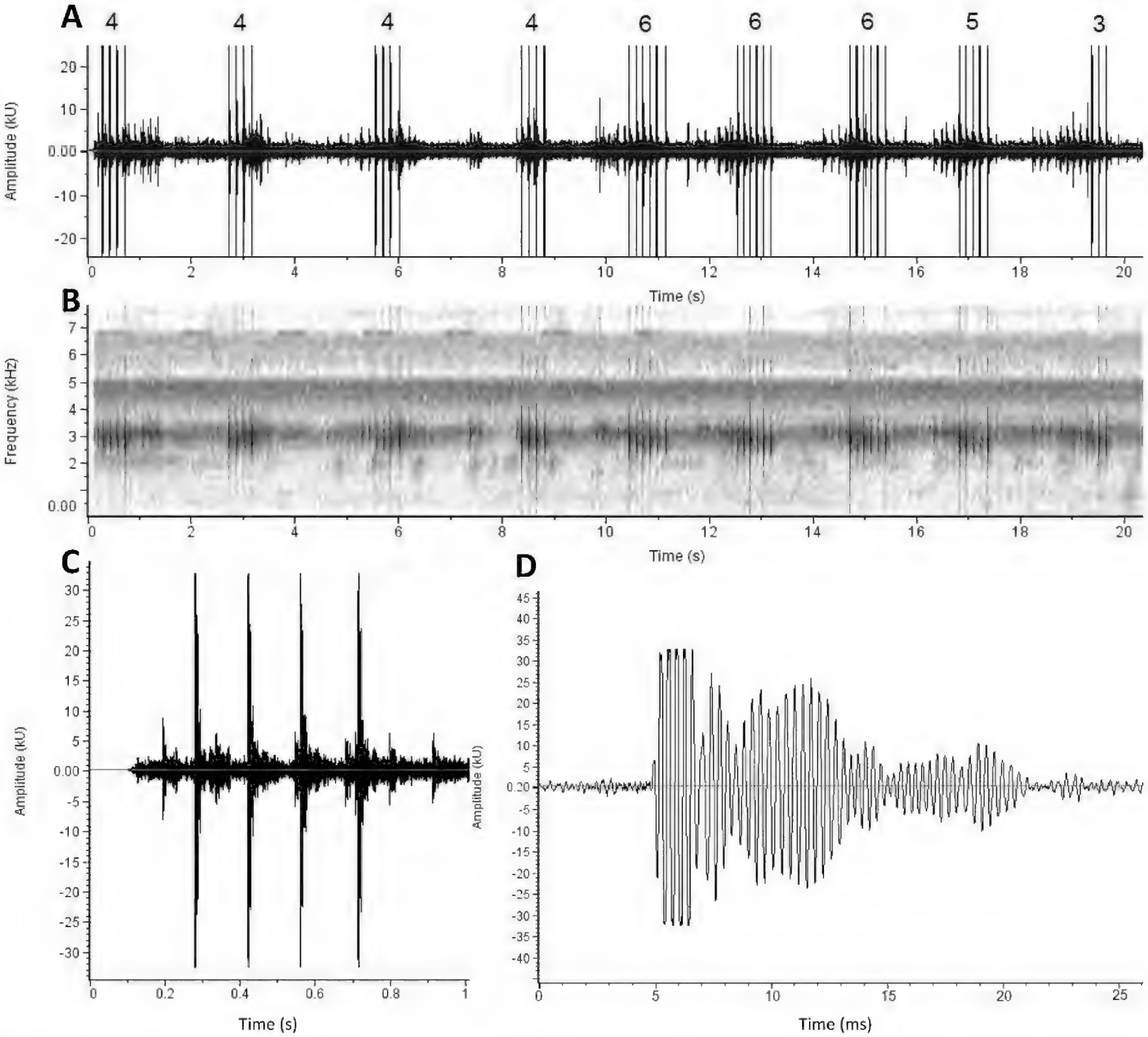

Fig 4. Advertisement call of *Raorchestes longchuanensis* at ambient air temperature 27.4 °C. (**A**) A call consists of five call groups, numbers on the top indicate pulse numbers in the respective call group. (**B**) Spectrogram of the call. (**C**) First call group with four pulses. (**D**) A pulse of the 5th call group.

new genus *Raorchestes* (based on molecular phylogeny), consisting of small frogs with no vomerine teeth, direct development without free swimming larvae, and a transparent gular pouch while calling (Biju et al. 2010).

At least 62 *Raorchestes* species have been named to date, but many of these are species complexes and further study could reveal cryptic species (Frost 2019). Many of the *Philautus* species from across the distribution of this genus have become subsumed into *Raorchestes* (Ghose and Bhuiyan 2012; IUCN 2015). This includes the former *P. annandalii* (Darjeeling Bush Frog) being changed to *R. annandalii*, also recorded from the northeastern regions of Bangladesh (IUCN 2015; Khan 2015, 2018). The second species, *R. parvulus* (Karin Bubble-nest Frog) reported by Mahony et al. (2009) and later by Ghose and Bhuiyan (2012), is considered to be a possible member of a species group found in southeastern Bangladesh (Mahony and Reza 2007a,b; Mahony et al. 2009; Khan 2015; IUCN 2016).

Raorchestes longchuanensis was previously considered endemic to southern China, restricted to some prefectures in Yunnan province at 1,150–1,600 m above sea level, adjacent to northern and northeastern Myanmar (Yang et al. 2004). However, this species has already been recorded from Vietnam (Orlov et al. 2002). Yang et al. (2004) have suggested that the species was likely to occur in neighboring countries including northern Myanmar, Thailand, and Laos. The species has escaped detection in some areas possibly due to its cryptic nature and the general absence of major exploration in these regions.

The findings reported here also point to the broader floral similarities between Myanmar and northeastern India (including states such as Tripura, Mizoram, Manipur, and Nagaland) [Slik et al. 2018], which forms a belt connecting our study site, and northern Myanmar across into Yunnan, China. This study presents a range extension of this species of approximately more than 600 km in the western direction from its type locality (Fig. 5). An earlier study showed a range extension of

Table 3. Intraspecific and interspecific genetic divergence. Uncorrected *p*-distance between 16S rDNA sequences of 20 closely related species of *Raorchestes* and *Kurixalus eiffingeri*.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1	*R. longchuanensis* (GQ285675)	--																											
2	*R. longchuanensis* (MH699074)	0.002	--																										
3	*R. longchuanensis* (MN193412)	0.005	0.002	--																									
4	*R. longchuanensis* (MN193413)	0.002	0.000	0.002	--																								
5	*R. longchuanensis* (MN193414)	0.002	0.000	0.002	0.000	--																							
6	*R. ghatei* (KF366385)	0.050	0.048	0.050	0.048	0.048	--																						
7	*R. gryllus* (AB933309)	0.050	0.048	0.050	0.048	0.048	0.045	--																					
8	*R. shillongensis* (MG980282)	0.075	0.073	0.075	0.073	0.073	0.061	0.055	--																				
9	*R. shillongensis* (MG980283)	0.075	0.073	0.075	0.073	0.073	0.061	0.055	0.000	--																			
10	*R. bombayensis* (KF767502)	0.059	0.057	0.059	0.057	0.057	0.043	0.045	0.070	0.070	--																		
11	*R. bombayensis* (EU450019)	0.059	0.057	0.059	0.057	0.057	0.043	0.045	0.070	0.070	0.000	--																	
12	*R. parvulus* (MH590204)	0.066	0.064	0.066	0.064	0.064	0.048	0.059	0.070	0.070	0.061	0.061	--																
13	*R. tuberohumerus* (KP137388)	0.061	0.059	0.061	0.059	0.059	0.041	0.048	0.068	0.068	0.020	0.020	0.064	--															
14	*R. beddomii* (EU449998)	0.077	0.075	0.077	0.075	0.075	0.066	0.080	0.091	0.091	0.080	0.080	0.080	0.073	--														
15	*R. chalazodes* (KJ619641)	0.105	0.102	0.105	0.102	0.102	0.089	0.091	0.098	0.098	0.082	0.082	0.077	0.084	0.059	--													
16	*R. aureus* (KM596540)	0.075	0.073	0.075	0.073	0.073	0.059	0.075	0.082	0.082	0.064	0.064	0.080	0.066	0.057	0.066	--												
17	*R. charius* (KU169985)	0.093	0.091	0.091	0.091	0.091	0.070	0.089	0.093	0.093	0.080	0.080	0.066	0.077	0.055	0.068	0.066	--											
18	*R. chromasynchysi* (KM596543)	0.086	0.084	0.086	0.084	0.084	0.061	0.084	0.080	0.080	0.075	0.075	0.068	0.086	0.052	0.055	0.050	0.057	--										
19	*R. duboisi* (JX092668)	0.086	0.084	0.086	0.084	0.084	0.066	0.068	0.080	0.080	0.068	0.068	0.073	0.075	0.043	0.048	0.052	0.061	0.043	--									
20	*R. indigo* (KM596557)	0.061	0.059	0.061	0.059	0.059	0.041	0.061	0.075	0.075	0.059	0.059	0.066	0.061	0.064	0.073	0.036	0.066	0.055	0.052	--								
21	*R. marki* (JX092719)	0.070	0.068	0.070	0.068	0.068	0.066	0.073	0.080	0.080	0.066	0.066	0.073	0.059	0.061	0.057	0.045	0.057	0.050	0.048	0.043	--							
22	*R. menglaensis* (EU924621)	0.059	0.057	0.059	0.057	0.057	0.050	0.057	0.068	0.068	0.059	0.059	0.011	0.061	0.082	0.084	0.082	0.068	0.070	0.075	0.064	0.070	--						
23	*R. montanus* (KM596552)	0.086	0.084	0.086	0.084	0.084	0.066	0.086	0.093	0.093	0.070	0.070	0.082	0.077	0.059	0.061	0.034	0.064	0.043	0.050	0.048	0.055	0.084	--					
24	*R. munnarensis* (DC092655)	0.091	0.089	0.091	0.089	0.089	0.070	0.082	0.086	0.086	0.080	0.080	0.082	0.073	0.041	0.057	0.057	0.068	0.048	0.023	0.061	0.048	0.084	0.055	--				
25	*R. ponmeidi* (KM596576)	0.068	0.066	0.068	0.066	0.066	0.057	0.066	0.093	0.093	0.073	0.073	0.075	0.075	0.055	0.089	0.057	0.070	0.068	0.064	0.050	0.066	0.077	0.064	0.066	--			
26	*R. ponmudi* (EU4511026)	0.068	0.066	0.068	0.066	0.066	0.057	0.066	0.093	0.093	0.073	0.073	0.075	0.075	0.055	0.089	0.057	0.070	0.068	0.064	0.050	0.066	0.077	0.064	0.066	0.000	--		
27	*R. signatus* (KM596561)	0.086	0.084	0.086	0.084	0.084	0.066	0.084	0.084	0.084	0.075	0.075	0.077	0.077	0.048	0.050	0.025	0.061	0.041	0.043	0.041	0.048	0.080	0.020	0.048	0.061	0.061	--	
28	*Kurixalus eiffingeri* (DQ468673)	0.139	0.141	0.139	0.141	0.141	0.141	0.141	0.148	0.148	0.132	0.132	0.157	0.134	0.130	0.139	0.148	0.141	0.132	0.136	0.148	0.134	0.155	0.148	0.132	0.145	0.145	0.141	--

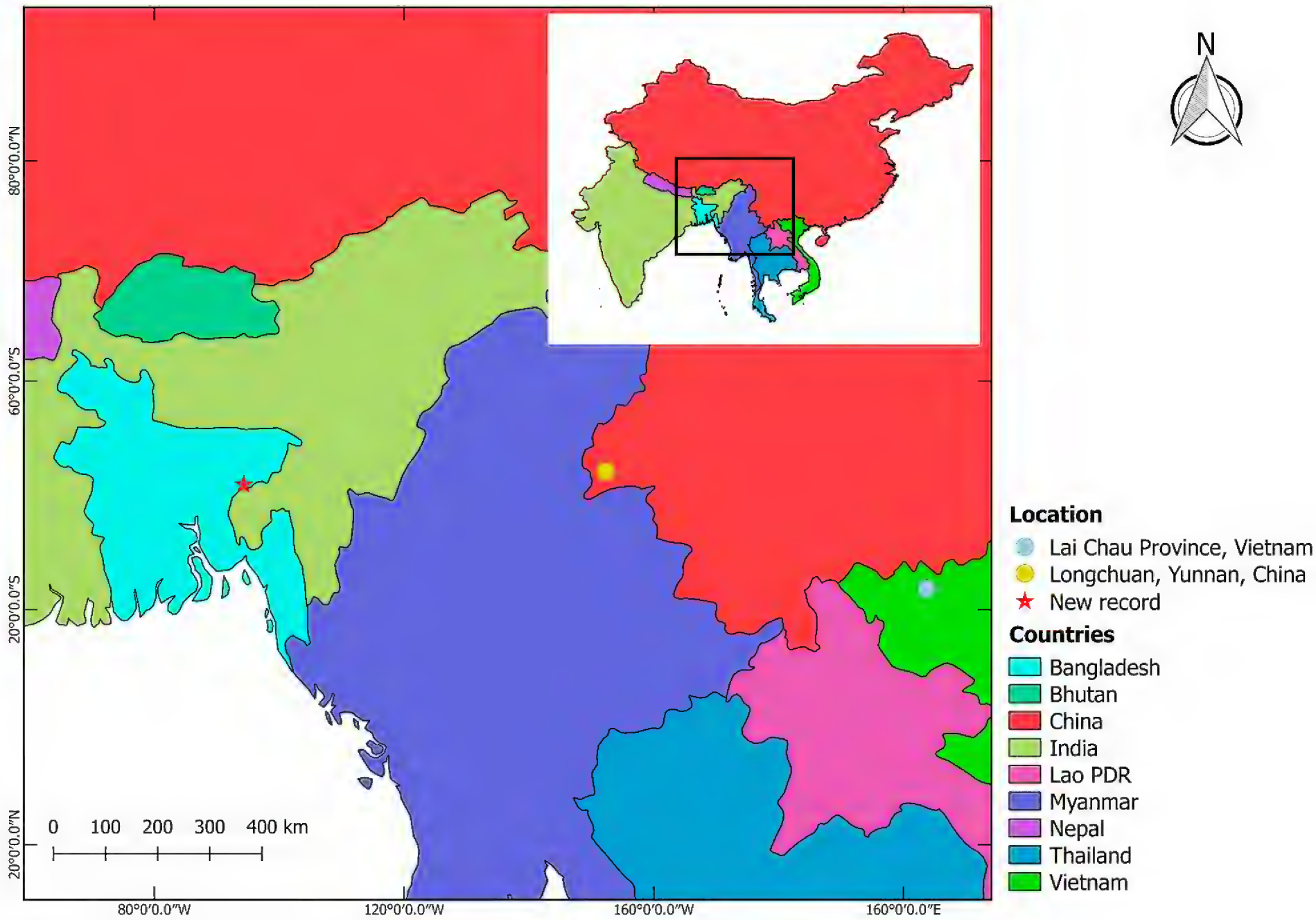

Fig 5. Map showing the previously reported and present records of *Raorchestes longchuanensis*.

more than 600 km in the east for this species (Orlov et al. 2002). Thus, we suspect that the species has a much wider distribution, and further studies in these regions will confirm its occurrence. We hope that more studies, especially those targeting arboreal species, will continue in Bangladesh and in neighboring regions to document and potentially save these species from the risks posed by the perpetual exploitation of timber in these regions. A comprehensive effort needs to be made to at least safeguard the remaining wilderness areas in Bangladesh and in neighboring regions.

Conclusions

This study adds *Raorchestes longchuanensis* as a new species to the anuran fauna of Bangladesh. Although currently assessed as Least Concern by the IUCN (Yang et al. 2004), its geographic distribution and current conservation status need to be re-evaluated, since its habitats have further degraded since the last assessment. The breeding biology, ecology, and habitat characteristics of the species need to be determined to ensure long-term conservation of this species in Bangladesh and elsewhere.

Acknowledgements.—We thank the Forest Department of Bangladesh for their support and facilitation of this work. The authors are thankful to Professor Dr. Abdul Alim, Chairman, Department of Zoology, Jagannath University, for his support. We are thankful to Md. Anisur Rahman (beat officer) and Mahbub Alam (range officer) of Satchari National Park for their support during the study. We are especially thankful to Tanvir Ahmed for his support during field work.

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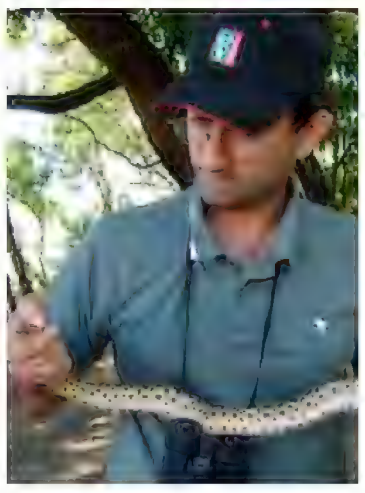

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